

# On the Live Coloration and Partial Mitochondrial DNA Sequences in the Topotypic Population of *Goniurosaurus kuroiwaie orientalis* (Squamata: Eublepharidae), with Description of a New Subspecies from Tokashikijima Island, Ryukyu Archipelago, Japan

Masanao HONDA<sup>1\*</sup> and Hidetoshi OTA<sup>2</sup>

<sup>1</sup> Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

<sup>2</sup> Institute of Natural and Environmental Sciences, University of Hyogo, and Museum of Nature and Human Activities, Sanda, Hyogo 669-1546, Japan

**Abstract** Careful investigations of live coloration and other characters in the eublepharid gecko *Goniurosaurus kuroiwaie orientalis* from Tonakijima Island, the type locality of the subspecies in the Central Ryukyus, Japan, revealed its slight but consistent external differences from the consubspecific Tokashikijima population, whose phylogenetically divergent status against the former had been strongly suggested in a previous molecular study. A new subspecies, *Goniurosaurus kuroiwaie sengokui* subsp. nov., is thus described for the Tokashikijima population. In life, *Goniurosaurus k. sengokui* subsp. nov. can be discriminated from *G. k. orientalis* in showing tint of reddish or pinkish, instead of yellowish, coloration in dorsal body pattern, and also usually darker and more or less reddish, instead of yellowish or grayish, iris in both adults and juveniles. Recalculation of sequence data at hand with relevant GenBank data confirmed substantial sequence divergences of *G. k. sengokui* from all other congeneric taxa of the Ryukyus described so far.

**Keywords** Eublepharidae, *Goniurosaurus*, morphology, taxonomy, Ryukyu Archipelago

## 1. Introduction

The eublepharid geckos of the genus *Goniurosaurus* (Reptilia: Squamata) are distributed in the southeastern part of continental Asia and adjacent continental-shelf islands, and the central part of the Ryukyu Archipelago, Japan. A total of 12 species are recognized from the former region (Orlov *et al.*, 2008; Wang *et al.*, 2010, 2013, 2014; Yang and Chan, 2015; Ziegler *et al.*, 2008), whereas species-level taxonomy of the Ryukyu populations, so-called *G. kuroiwaie* group (Grismer *et al.*, 1999) in highly relict state (Okamoto, 2017; Ota, 1998), is somewhat controversial (see below).

By the 1950s, four eublepharid geckos had been described from the Ryukyu Archipelago: *Gymnodactylus albofasciatus kuroiwaie* Namiye, 1912 from Okinawajima

Island, *Eublepharis orientalis* Maki, 1930 from Tonakijima Island, *Gymnodactylus yamashinae* Okada, 1936 from Kumejima Island, and *Eublepharis splendens* Nakamura and Uéno, 1959 from Tokunoshima Island. In their comprehensive taxonomic synthesis of the Japanese herpetofauna, Nakamura and Uéno (1963) rearranged these taxa with an addition of one local population into three conspecific subspecies: *E. k. kuroiwaie* from Okinawajima, *E. k. orientalis* from Tonakijima, Kumejima and Tokashikijima, and *E. k. splendens* from Tokunoshima. Most subsequent authors followed this classification (e.g., Sengoku, 1979; Toyama, 1984; Wermuth, 1965) until the publication of Grismer (1987), who removed all East Asian eublepharids from *Eublepharis*, a genus originally described with *Eublepharis hardwickii* Gray, 1827 from India as the type species, and reassigned them to the resurrected genus *Goniurosaurus* Barbour, 1908. Although Grismer (1987) did not recognize subspecies in *G. kuroiwaie*, he later revalidated “*yamashinae*” and “*splendens*”

\* Corresponding author: Dr. Masanao HONDA, from University of Tsukuba, Tsukuba, Ibaraki, Japan, with his research focusing on systematics and conservation genetics of reptiles in Japan.  
E-mail: honda.masanao.ge@u.tsukuba.ac.jp  
Received: 6 January 2017 Accepted: 25 April 2017

as its subspecies (Grismer, 1988). Based on detailed morphological analyses of specimens representing almost all populations of *G. kuroi* *sensu lato* known to that date, Grismer *et al.* (1994) re-classified the Ryukyu populations into five conspecific subspecies: *G. k. kuroi* from Okinawajima, Sesokojima and Kourijima (see Kurita and Kawamura [2011] for distribution of Yagajijima), *G. k. orientalis* from Tonakijima, Tokashikijima, Akajima and Iejima, *G. k. yamashinae* from Kumejima, *G. k. toyamai* from Iheyajima, and *G. k. splendens* from Tokunoshima (Figure 1). Grismer *et al.* (1999) elevated these five taxa to full species from the viewpoint of evolutionary species concept, whereas Ota *et al.* (1999) maintained Grismer *et al.*'s (1994) classification in recognition of its greater phylogenetic informativeness and adequacy for apparently poor morphological divergences among the five taxa.

Recently, Honda *et al.* (2014) provided results of detailed molecular analyses among most populations of *G. kuroi* *sensu lato* that showed substantial incongruence with their current taxonomic arrangement. In that study, particularly large genetic divergence was elucidated between *G. k. splendens* of Tokunoshima of the Amami Island Group and the other subspecies all from islands belonging to the Okinawa Island Group, and the former was thus accorded full species status, the treatment that had already been practiced by Grismer *et al.* (1999) on the basis of a different paradigm (see above). Honda *et al.* (2014) also detected apparent non-monophyly and substantial differentiations, though not so large as those between the Tokunoshima and the other populations, among populations assigned to *G. k. orientalis*. Of these, the non-monophyly of this subspecies was due to much closer genetic affinities of its Iejima population to populations of the nominotypical subspecies on Okinawajima than to other “conspecific” (“*orientalis*”) populations, whilst distinct genetic divergence was also recognized between two of the remaining “*orientalis*” (i.e., Tonakijima and Tokashikijima) populations (see Remarks in Taxonomy for the population status of the Akajima “*orientalis*”). However, Honda *et al.* (2014) deferred the apparent problematic taxonomic arrangement relevant to *G. k. orientalis* for future studies due to an almost complete absence of information regarding morphological variation within the subspecies.

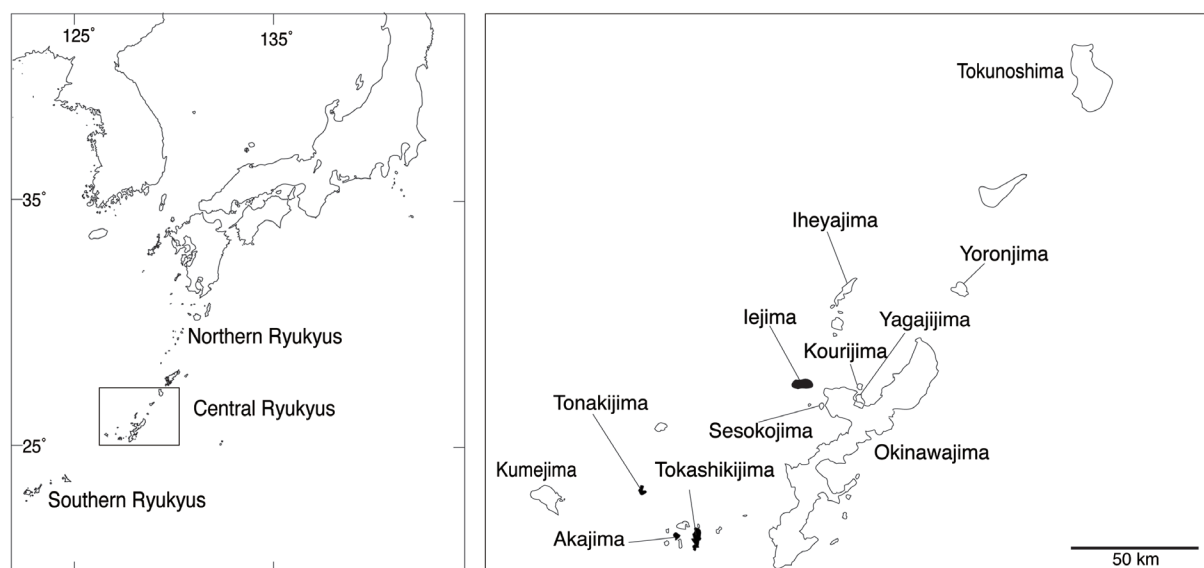
During our recent field works on Tonakijima (type locality of *G. k. orientalis*) and Tokashikijima, we observed live representatives of the two island populations, which revealed their substantial differences. Such differences have never been mentioned by the

previous authors (e.g., Grismer *et al.*, 1994; Nakamura and Uéno, 1963; Sengoku, 1979), because none of them examined topotypic specimens in life; the holotype and one juvenile, both long preserved and thus with much faded coloration have long been the only Tonakijima specimens available to researchers (Ota, 1989, 1997). Hence, we herein describe a new subspecies for the Tokashikijima population on the basis of our novel findings.

## 2. Materials and Methods

We examined 38 specimens (21 males and 17 females, all from Tokashikijima, National Science Museum, Tokyo, Herpetological Collection [NSMT-H] 02523–02524, Okinawa Prefectural Museum, Herpetological Collection [OPM-H] 1502500, 1502505–1502541) preserved in 70% ethanol. We also examined the holotype of *Goniurosaurus kuroi orientalis* (= *Eublepharis orientalis* Maki, 1930: NSMT-H 02522), and another preserved specimen from Tonakijima (juvenile, OPM-H 489). The holotype was formerly located in the “Zoological Institute, College of Science, Kyoto Imperial University” (= Department of Zoology, Graduate School of Science, Kyoto University at present). Maki (1930) sexed the holotype as female, but it actually has well developed testes and thus is an adult male (Nakamura and Uéno, 1963; Ota, 1997). The unique juvenile from Tonakijima was in rather poor preservation state, and quantitative data from this specimen used for comparisons were thus limited to only four of the nine meristic characters mentioned below (Table 1).

Measurements were taken to nearest 0.1 mm with dial calipers for lengths of snout to vent (SVL), snout to ear-opening (S-EO), snout to anterior border of eye (S-E), posterior border of eye to ear-opening (E-EO), forelimb (FL) and hindlimb (HL), and maximum width of head (HW). In addition, nine meristic characters were examined, of which eight followed Grismer's (1988) definitions. These were: supralabials (SL), the series posterior to the rostral and terminating with a scale at least twice size of the surrounding granular scales; infralabials (IL), the series posterior to the mental and terminating with a scale at least twice the size of the surrounding granular scales; postmental (PM), all scales except the first infralabials which contact the mental; preoculars (PO), the linear arrangement of granular scales between the anterior corner of the eye and the posterior margin of the external nares; eyelid fringe scales (EF), the lateralmost enlarged triangular scales encircling the eye; midbody scales (BO), number of granular scales



**Figure 1** Left: Map of East Asia showing the range of distribution of *Goniurosaurus kuroi* sensu lato and *G. splendens* (indicated by square). Right: Magnified map of the area enclosed in the above square showing locations of islands, on which *G. k. orientalis* sensu Grismer *et al.* (1994) occurs (shaded ones). In the latter map, names of islands, from which other members of *Goniurosaurus* in the Ryukyu Archipelago have been recorded to the present (Grismer *et al.*, 1994; Kurita and Kawamura, 2011; Maenosono and Toda, 2007; Nakamura *et al.*, 2014), are also provided.

surrounding the body midway between limb insertion; fourth toe lamellae (4T), counting from the union of the fourth toes and terminating with the distal penultimate scale; claws scales (CL) surrounding claw on fourth toe. For the number of paravertebral tubercles (TU), we counted enlarged and thickened scales from the occipital region to the base of tail instead of those between limb insertions as in Grismer (1988), because discontinuity of such tubercles from other yet more or less enlarged scales were much more prominent in those regions than in the regions above limb insertions. As to bilateral characters, we measured the right side in principle.

Sexual difference in SVL and meristic characters were examined by student's t-test and Mann-Whitney U test, respectively. Analyses of covariance (ANCOVA) using SVL as a covariate were used to assess the difference between males and females in other morphometric characters. Characters were also analyzed by principal component analysis (PCA) using correlation matrix. All analyses were conducted using R 3.1.0 (R Development Core Team, 2011), setting significance levels at 0.05.

We also conducted field surveys on Tonakijima in 2011 and 2016, and observed a total of three adults and three juveniles there. Also, we carried out additional field works on Tokashikijima in 1999, 2010 and 2016, and observed a total of 17 adults and 10 juveniles. On Tonakijima, colorations of adults were observed and their dorsal views were photographed without handling, whereas the juveniles were captured under the due permission,

photographed, and released. On Tokashikijima, nine adults and eight juveniles, encountered during the fieldwork in 1999, were directly checked for character states in coloration to confirm their identity to *G. k. orientalis* as defined by Grismer *et al.* (1994). Coloration for other Tokashikijima individuals (eight adults and two juveniles) were recorded by the same way as for the Tonakijima individuals mentioned above.

For genetic and phylogenetic analyses, we incorporated our published sequence data for *G. kuroi* sensu lato (Honda *et al.*, 2014; Ota *et al.*, 1999) and comparable data for representative samples of two Chinese-Vietnamese congeners, *G. lui* Grismer *et al.*, 1999 and *G. huiliensis* Orlov *et al.*, 2008, available in GenBank (AB853424–AB853436, AB853441–AB853453, and AB853458–AB853479).

The Iejima population is assigned to *G. k. orientalis* by previous studies (e.g., Grismer *et al.*, 1994; Nakamura and Uéno, 1963). However, recent molecular analyses suggest this population is genetically closer to populations assigned to the nominotypical subspecies (Honda *et al.*, 2014; Ota *et al.*, 1999). Thus, we referred to the Iejima population as *G. k. cf. kuroi* in Results. Nakamura *et al.* (2014) described a putatively recently extinct subspecies, *G. k. yunnu*, on the basis of skeletal remains recovered from a midden on Yoronjima. However, type series of this subspecies showed several morphological differences from skeletal specimens of other *G. kuroi* populations including those from Tokashikijima (referred

to as *G. k. orientalis*, then: see Nakamura *et al.* [2014] for further details). We thus exclude this subspecies from further comparisons below.

### 3. Results

#### 3.1 Comparisons between the Tokashikijima and Tonakijima specimens

##### 3.1.1 Redescription of holotype of *G. k. orientalis*

Head large, somewhat rhomboidal outline, covered with granular scales, intermixed with enlarged subconical tubercles on occiput; rostral subpentagonal, approximately twice as wide as high; present 19 PO; E-EO slightly shorter than S-E; present nine SL and 10 IL; mental nearly trigonal and large; present three PM; eyelids well developed and movable; present 48 EF and 45 TU; body with minute scales, intermixed with numerous conical tubercles on the dorsal and lateral sides; ventral surfaces of body with granular minute scales; limbs long, covered with minute granular scales similar to those on the body; digits not very long rather stout, moderately compressed; present 21 4T and six CL; tail swollen, rounded. Coloration including transverse bands and spots on body of the holotype almost completely faded (Figure 2 A). Other measurements were presented in Table 1.

**3.1.2 Live coloration of Tonakijima specimens** Dorsal ground color of live individuals of Tonakijima dark blackish brown; two of the three adults observed with ochereous yellow, partially broken reticulation on head; distinct ochereous yellow middorsal stripe beginning from nape, extending only to the first transverse band of same color at position of forelimb insertions; the first band, extending on dorsal surfaces of upper-arms to elbows on both sides, and three additional ochereous yellow bands with the last one being above posterior margin of hind-limb insertions. Tail apparently intact in one of these two adults, damaged (half-autotomized) in the other; ground color of intact tail also dark blackish brown, with five white annuli. Interspaces between first and second, second and third, and third and fourth bands on body, as well as those between fourth band on body and first annulus on tail, and first and second annuli on tail, with areas of light mottling, looking like additional but more or less indistinct transverse bands (Figure 3 A).

All three juveniles observed on Tonakijima were similar to the two adults described above in dorsal coloration except for brighter yellow in transverse bands, indistinct border between dark- and light-colored regions on the anterodorsal surface of head, presence of an additional transverse yellow band on the nape, and

interruption of the middorsal stripe by light mottling between the nape and the first transverse body band at the position of forelimb insertions (Figure 3 C).

Dorsal coloration in the remaining adult observed on Tonakijima was slightly different from that in any of the other four Tonakijima individuals by having yellow, instead of ochereous yellow, pattern on dorsal surfaces of head and body, which consisted of three more or less prominent transverse bands in the occipitonuchal region, two incomplete bands on anterodorsal surface of body, and five complete and nearly complete bands on the mediodorsal and posterodorsal surfaces of body. Middorsal stripe was broken into three portions; one from the nape to the nuchal band, another from the middle of the interspace between the nuchal band and first incomplete body band to the region slightly posterior to the latter, and from the middle of the interspace between the first and second incomplete body bands to the region slightly posterior to latter. There was a slight but still discernible region with light mottling in each of the interspaces between those seven incomplete and complete bands on body (Figure 3 B). In all six live individuals on Tonakijima, iris was brown with tint of yellowish gray.

##### 3.1.3 Live coloration of Tokashikijima specimens

Observations of live individuals on Tokashikijima largely confirmed descriptions in Grismer *et al.* (1994): dorsal ground color dark blackish brown, with more or less reddish or pinkish tint of orange dorsal pattern consisting of four somewhat indistinct transverse bands and middorsal stripe from the nape at least to the first transverse band in adults; more distinct transverse bands in similar color in juveniles (Figure 3 D, E). In most individuals observed alive, iris was red (Figure 3 F), but that of a few juveniles were more yellowish than of others, although it still retained some tint of reddish coloration there.

##### 3.1.4 Comparisons of morphometric and meristic characters

In each of the morphometric and meristic characters examined exclusive of the snout-vent length (SVL) in adult and the number of lamellae on the fourth toe (4T), values in the two preserved Tonakijima specimens fell within the range of corresponding values in the preserved Tokashikijima specimens (Table 1, see Taxonomy for further details). Although we examined only one adult specimen of the Tonakijima population and thus failed to take any quantitative comparisons, we suspect that the Tokashikijima population tends to have smaller body measurements and fewer lamellae on fourth toe than the Tonakijima population.

##### 3.1.5 Comparisons of color in life

Color in life of dorsal





**Figure 2** Dorsal views of *Goniurosaurus kuroiwan* specimens in ethanol. A: holotype of *G. k. orientalis* (NSMT-H 02522). B: holotype of *G. k. sengokui* subsp. nov. (NSMT-H 10808). Paratype males (C, OPM-H 1502528, 1502529, 1502531, 1502527 and 1502518) and females (D, OPM-H 1502521, 1502534, 1502519, 1502535 and 1502520) of *G. k. sengokui* subsp. nov. showing variation in head, body, and tail patterns.



**Figure 3** Dorsal views of live *Goniurosaurus kuroiwan* observed on Tonakijima (A and B, adults; C, juvenile) and Tokashikijima (D, adult; E, juvenile; F, eye color of adult).

body pattern and iris almost completely differentiated the Tokashikijima population from the topotypic (Tonakijima) population of *G. k. orientalis*, because the latter lacked reddish or pinkish tint in color of body pattern and of iris (Figure 3), despite the broadly believed presumption for this subspecies that had been created largely on the basis of comparative studies chiefly using specimens from Tokashikijima (e.g., Grismer *et al.*, 1994).

**3.2 Intersexual comparisons** In the Tokashikijima population, statistically significant differences between sexes were recognized in EF and HW (Table 1). The PCAs for all (Figure 4) and morphometric characters (not shown) revealed 73.4% and 98.4% of the total variation expressed in the first two principal components (PRIN1 and PRIN2), respectively. Scatter plots of scores on PRIN1 and PRIN2 could not separate males and females. The plot of the unique adult male from Tonakijima (holotype of *G. k. orientalis*) was separated from those of the Tokashikijima specimen.

### 3.3 Genetic divergences among island populations

Twenty-five haplotypes consisting of a total 1942 base positions of 12S and 16S rRNAs, and cytochrome *b* genes were reanalysed on the basis of previously published sequence data in Ota *et al.* (1999) and Honda *et al.* (2014). The fragment of these three genes consisted of 402, 466, and 1074 bp, respectively. Inter-specific nucleotide divergence between two Chinese-Vietnamese congeners (*G. luii* vs. *G. huuliensis*) was 2.7% (uncorrected *p*-distance), whereas 4.5% difference was observed between samples from Tokashikijima and Tonakijima (Table 2). Also, the Tokashikijima sample exhibited relatively large divergences with samples from Tokunoshima (*G. splendens*, 10.5%), Iheyajima (*G. k. toyamai*, 4.7%), the northern part of Okinawajima (*G. k. kuroiwaie* [part], 5.7%), the southern part of Okinawajima (*G. k. kuroiwaie* [part], 4.6%), Iejima (*G. k. cf. kuroiwaie*, 4.4%), and Kumejima (*G. k. yamashinae*, 4.3%).

### 3.4 Taxonomy

***Goniurosaurus kuroiwaie sengokui*, subsp. nov. (Figures 2 B, 5)**

#### Synonymies

*Eublepharis kuroiwaie orientalis*: Nakamura and Uéno, 1963 (part).

*Eublepharis kuroiwaie orientalis*: Sengoku, 1979 (part).

*Amamisaurus orientalis orientalis*: Börner, 1981 (part).

*Eublepharis kuroiwaie orientalis*: Toyama *et al.*, 1984 (part).

*Goniurosaurus kuroiwaie*: Grismer, 1987 (part).

*Goniurosaurus kuroiwaie kuroiwaie*: Grismer, 1988 (part).

*Goniurosaurus kuroiwaie orientalis*: Ota, 1989 (part).

*Goniurosaurus kuroiwaie orientalis*: Toyama and Ota, 1991 (part).

*Goniurosaurus kuroiwaie orientalis*: Grismer *et al.*, 1994 (part).

*Goniurosaurus kuroiwaie orientalis*: Tanaka, 1996 (part).

*Goniurosaurus orientalis*: Grismer, 2002 (part).

*Goniurosaurus orientalis*: Dickhoff, 2004 (part).

*Goniurosaurus kuroiwaie orientalis*: Werner *et al.*, 2004 (part).

*Goniurosaurus kuroiwaie orientalis*: Tanaka, 2005 (part).

*Goniurosaurus orientalis*: Seuffer *et al.*, 2005 (part).

*Goniurosaurus orientalis*: Ziegler *et al.*, 2008 (part).

*Goniurosaurus kuroiwaie orientalis*: Ota, 2010 (part).

**Holotype** NSMT-H 10808 (formerly Satoshi Tanaka's Private Collection [TPN] 78080509 listed in Grismer *et al.* [1994]), an adult male, collected from Tokashiki (ca. 26.2 N, 127.4 E), Tokashikijima, Okinawa Island Group, Central Ryukyus, Japan in 5th August 1978, collected by Satoshi Tanaka.

**Paratypes** Thirty-seven paratypes from Tokashikijima, of which the latter 35 were collected from the same locality as the holotype. NSMT-H 02523 (adult male), 02524 (adult female), OPM-H 1502506–1502507, 1502509–1502514, 1502516–1502518, 1502527–1502532, 1502527 (adult males), 1502500, 1502505, 1502508, 1502515, 1502519–1502526, 1502533–1502538 (adult females) (formerly Satoshi Tanaka's Private Collection [TPN] 78052001–78052002, 78052101–78052106, 78052108–78052109, 78052201, 78080501, 78080508, 78080512–78080515, 79082602, 77120703, 78030103, 78052003, 78052107, 78060501, 78061101, 78061401, 78061601, 78071101, 78071201, 78071901, 78072003, 78090202–78090205, 79082603).

**Etymology** The subspecific epithet, “*sengokui*”, noun in genitive case, derives from the last name of late Mr. Showichi Sengoku, honoring his great contribution to the enhancement of social awareness and appreciation of wildlife including reptiles in Japan.

**Distribution** *Goniurosaurus k. sengokui* subsp. nov. is currently known only from Tokashikijima, Okinawa Island Group, Ryukyu Archipelago, Japan (Figure 1). It seems to have been distributed in Akajima (see Remarks).

**Diagnosis** *Goniurosaurus k. sengokui* subsp. nov. is distinguishable from all other Ryukyu taxa of the genus as follows. It differs from *G. k. yamashinae* in showing lineate tendency of middorsal patterns at least in the anterior body, darker, more or less reddish iris and dorsal pattern; from *G. splendens* also in showing



**Table 1** Comparisons of morphometric and meristic characters among adult males and adult females of *Goniurosaurus kuroiwae sengkui* subsp. nov., and the two specimens (one adult male [holotype] and one juvenile) of *G. k. orientalis* sensu stricto. SVL: snout-vent length; S-E: snout to ear-opening length; S-EO: snout to anterior border of eye length; E-EO: ear-opening to posterior border of eye length; HW: head width; FL: forelimb length; HL: hindlimb length; SL: number of supralabials; IL: number of infralabials; PM: number of postmentals; PO: number of preoculars; EF: number of enlarged fringe scales on the eye; TU: number of paravertebral tubercles; BO: number of midbody scale row; 4T: number of fourth toe lamellae; and CL: number of scales surrounding claw on fourth toe.

Sample	SVL	S-EO	S-E	E-EO	HW*	FL	HL	SL	IL	PM	PO	EF*	TU	BO	4T	CL
<i>G. k. sengkui</i> subsp. nov.																
Male ( <i>n</i> = 21)																
average	74.8	17.7	7.6	6.7	13.4	24.4	31.8	9.8	9.2	3.9	23.4	54.3	46.0	140.2	18.7	6.0
range	49.6–88.3	11.6–21.2	5.4–8.7	3.9–8.1	9.1–15.9	15.4–28.7	21.8–37.4	8–11	8–11	3–5	20–51	43–62	39–51	131–157	17–20	6
Female ( <i>n</i> = 17)																
average	84.3	19.3	8.4	7.4	14.3	27.2	34.9	9.6	9.4	3.8	21.9	51.2	47.7	142.9	18.6	5.9
range	58.5–92.2	14.4–21.6	6.9–9.3	4.9–8.6	10.7–15.9	18.4–31.2	24.5–38.8	9–11	8–11	3–5	19–24	23–57	35–55	124–155	17–20	5–6
<i>G. k. orientalis</i> sensu stricto																
Male ( <i>n</i> = 1)																
	94.4	23.4	9.9	9.2	17.8	31.5	39.1	9	10	3	19	48	45	134	21	6
Juvenile ( <i>n</i> = 1) <sup>#</sup>																
										4				140	20	6

\* Differences in these characters between male and female *G. k. sengkui* subsp. nov. were statistically significant ( $P < 0.05$ ); those in other characters were not significant ( $P > 0.05$ ).

<sup>#</sup> This specimen was examined for only four meristic characters due to the poor preservation state (see text).

**Table 2** Pairwise *p*-distances (%) difference between samples of *Goniurosaurus kuroiwae* sensu lato and *G. splendens*. We reanalyzed a total 1942 base positions of 12S and 16S rRNA and cytochrome *b* genes based on published sequence data by Ota *et al.* (1999) and Honda *et al.* (2014).

Sample	1	2	3	4	5	6	7	8
1. Tokashikijima ( <i>G. k. sengkui</i> subsp. nov.)		10.5	4.7	5.7	4.6	4.4	4.5	4.3
2. Tokunoshima ( <i>G. splendens</i> )			10.8	11.5	10.2	10.3	11.0	10.9
3. Iheyajima ( <i>G. k. toyamai</i> )				5.4	5.4	4.3	4.4	4.9
4. Northern Okinawajima ( <i>G. k. kuroiwae</i> )					5.3	5.0	5.7	5.2
5. Southern Okinawajima ( <i>G. k. kuroiwae</i> )						1.2	4.6	4.3
6. Iejima ( <i>G. k. cf. kuroiwae</i> )							4.8	4.1
7. Tonakijima ( <i>G. k. orientalis sensu stricto</i> )								4.8
8. Kumejima ( <i>G. k. yamashinae</i> )								

lineate tendency of middorsal patterns at least in the anterior body, as well as mottlings in interspaces between successive transverse body bands, and by lacking sharply keeled tubercles, juxtaposed ventrals and the posteriormost body band extending onto hindlimbs; from *G. k. toyamai* also in showing lineate tendency of middorsal patterns at least in the anterior body, as well as mottlings in interspaces between successive transverse body bands, and by lacking robust body; from *G. k. kuroiwae* in having dorsal bands; and from *G. k. orientalis* in showing more or less reddish or pinkish tint in live coloration of dorsal body pattern and iris (Figure 2 D–F).

**Description of holotype** Adult male; SVL: 80.6 mm; tail length, from vent to tip of tail: 63.9 mm; S-EO: 18.4 mm; S-E: 7.5 mm; E-EO: 7.2 mm; HW: 14.1 mm; FL: 24.5 mm; and HL: 24.5 mm.

Head large, somewhat rhomboidal outline and covered with granular scales, intermixed with enlarged subconical tubercles on occiput; tubercles absent from rostrum to temple, but present posterior part of eye; dorsal tubercles usually smaller than the interspaces between them (Figure 5 A). Rostral subpentagonal with a median cleft above, approximately twice as wide as high, bounded above by two large nasals, two large supranasals and small scales between supranasals; nostril between nasal and supranasal, bounded dorso-posteriorly by eight (right) and eight (left) minute scales; present 22 (right) and 22 (left) preoculars from eye to nasal (Figure 5 B); distance between posterior border of eye and ear-opening slightly shorter than that from snout to anterior border of eye; present eight (right) and 11 (left) supralabials and as many sublabials, decreasing in size posteriorly; nine (right) and nine (left) infralabials and as many sublabials, decreasing in size posteriorly; mental nearly trigonal and large, much larger than first sublabial, in contact with four small postmentals (Figure 5 C); snout relatively narrow and sub-triangular; eyes large; eyelids well developed and movable, each with a fringe of conical scales at the base of eyelid; dorsal eyelid longer than ventral ones; present 56 (right) and 59 (left) enlarged conical fringe scales on the eye.

Neck narrower than body with minute scales, intermixed with numerous conical or trihedral tubercles on the dorsal and lateral sides; ventral surfaces with granular minute scales; no tubercles on ventral surfaces. Body elongate, somewhat slender, with long limbs; minute scales on the dorsal and lateral surface intermixed with numerous conical tubercles; dorsal and lateral tubercles usually smaller than the interspaces between them; ventral scales much larger and more or less flatter

than dorsal and loosely imbricate, juxtaposed on the greater part of belly except for the small area before vent; small area before vent with smaller scales (Figure 5 D); present 148 scales in a transverse row at midbody; from occiput to base of tail, present 51 paravertebral tubercles, of which 34 between the limb insertions. Limbs long and relatively slender, covered with minute granular scales similar to those on the body; many conical tubercles on their dorsal sides; digits not very long rather stout, moderately compressed and gradually narrowing towards apices; fourth toe with 20 (right) and 20 (left) small transverse lamellae below; claws short, sharp, strongly compressed and triangular; six (right) and six (left) claws surrounding claw on fourth toe.

Tail swollen, rounded, tapering to the distal end, above with small flat scales, beneath with larger flat imbricated scales; eleven series of tubercles on dorsum and lateral, arranged longitudinally; regenerated part of tail absent tubercles, but present flat scales.

Ground color of dorsum brown; head light brown to brown; cream white partially broken reticulation on head; labial region scales cream white; cream white middorsal stripe beginning from nape, extending to the first transverse band; four cream white transversal bands on the dorsal side of body; the foremost transversal band situated on forelimb, narrowest at both side of nape, fusing the middorsal stripe; the second band placed at anterior one-fourths of axilla to groin; the third band placed at anterior three-fourths of axilla to groin; the fourth band placed just behind hindlimbs; dorso-lateral cream white streaks on each side; cream white irregular spots on dorsal side of body. Forelimbs brown with cream white irregular pink spots or bands on dorsal side; hindlimbs brown with irregular cream white spots or bands on dorsal side. Tail brown with three cream white annuli; regenerated region of tail brown with cream white mottlings.

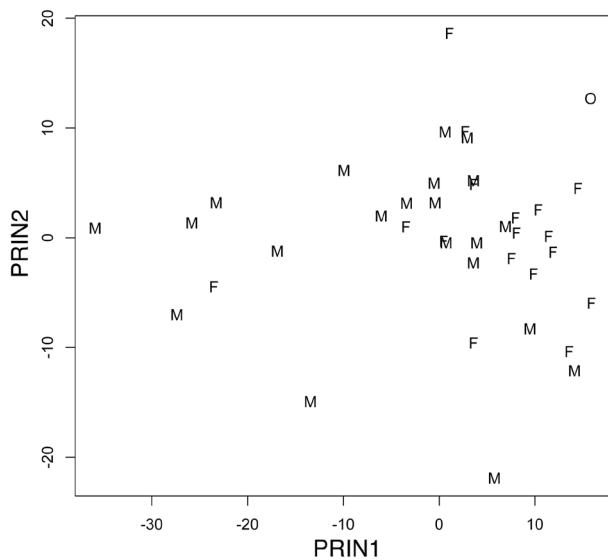
#### Variation of morphometric and meristic characters

Paratypes closely approximate the holotype in morphometric and meristic characters (Table 1), although some individuals lack a median cleft on rostral.

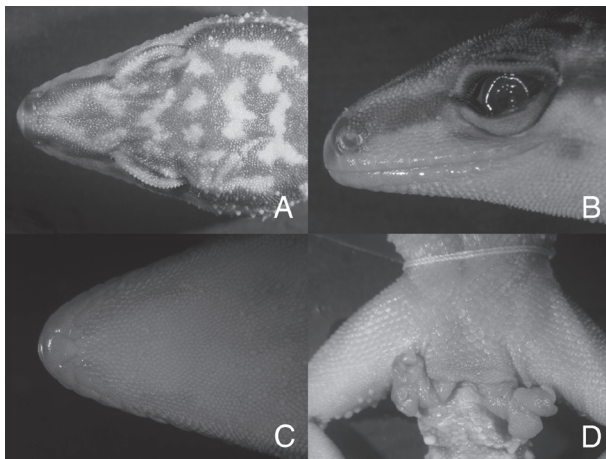
Males tend to have more eyelid fringe scales and broader head relative to SVL than females, but other characters overlapped and resembled each other (Table 1 and Figure 4).

**Color and variation of banding pattern** Ground color of dorsum in life, including limbs, dark blackish brown (Figure 3 D); cream white on the ventral side; more or less reddish or pinkish tint of orange, partially broken reticulation on head; infraorbital region from nostril to





**Figure 4** Plots of the first two principal component scores from morphological data for males (M) and females (F) of *Goniurosaurus kuroiwaie sengokui* subsp. nov., and male *G. k. orientalis* sensu stricto (O).



**Figure 5** Holotype of *Goniurosaurus kuroiwaie sengokui* subsp. nov. (NSMT-H 10808). A: dorsal view of head; B: lateral view of head; C: ventral view of head; and D: ventral view of the cloacal region.

ear-opening pale pinkish orange; labial region scales cream white; reddish or pinkish orange middorsal stripe beginning from nape, extending to the transverse band(s); more or less lineate tendency in band patterns, some with four reddish or pinkish orange transversal bands on the dorsal side of body, others with middorsal stripe instead of the band(s) especially in anterior part (Figure 2 C, D); the foremost transversal band situated on forelimb, narrowest at both side of nape, often invisible fusing the middorsal stripe; the second band placed at anterior one-fourths of axilla to groin, sometimes bended; the third band placed at anterior three-fourths of axilla to groin, sometimes

bended; the fourth band placed just behind hindlimbs; some with dorso-lateral reddish or pinkish orange streaks on each side; reddish or pinkish orange irregular spots on dorsal side of body (Figure 3 D). Forelimbs dark blackish brown with reddish or pinkish orange irregular pink spots or bands on dorsal side; hindlimbs dark blackish brown with irregular reddish or pinkish orange spots or bands on dorsal side. Tail dark blackish brown with some white annuli; regenerated region of tail dark blackish brown above and below, with scattered marble white spots or annuli. More distinct transverse bands in similar color in juveniles (Figure 3 E). Iris red in most individuals (Figure 3 F), but more yellowish color with some tint of reddish coloration in a few juveniles.

Ground color of dorsum in ethanol brown or dark brown; head and limbs light brown or brown; ground colors getting paler due to aged deterioration; bands, strips, reticulation and spots cream white (Figure 2 C, D).

Dorsal patterns including transverse bands are extremely variable (Figure 2 C, D). Most remarkable variation lies in the degree of lineate tendency in the middorsal pattern, such as no transversal band (leftmost in Figure 2 C), half band (middle in Figure 2 C, rightmost in Figure 2 D), extreme distortion (middle in Figure 2 C, second to the right in Figure 2 D), and thick dorso-lateral streaks on each side (rightmost in Figure 2 C).

**Natural history** *Goniurosaurus k. sengokui* is reportedly nocturnal and cursorial. It usually exhibits wide foraging for insects and other invertebrates on the ground and up to 1.8 m on trees, with or without occasional fairly long pausing (Werner *et al.*, 2004). Females with two oviductal eggs were recorded from early June to middle July (Tanaka, 2005).

**Remarks** Judging from external features including coloration of the unique representative specimen from Akajima (Grismer *et al.*, 1994; Toyama, 1983), the population seems to belong to *G. k. sengokui* subsp. nov. (see Discussion for current status of the Akajima population).

#### 4. Discussion

In the classification of *Goniurosaurus kuroiwaie* sensu lato, Grismer *et al.* (1994) used several characters in coloration, of which two can be observed only in live animals – color of juvenile dorsal pattern in life and color of iris in life. States of these characters for *G. k. orientalis* in Grismer *et al.* (1994) were determined chiefly by observing live specimens from Tokashikijima; their states in the topotypic (Tonakijima) population of

the subspecies were tentatively estimated on the basis of observations of the two long preserved specimens (see Materials and Methods and Results) and a very brief field-sighting of one juvenile in the dawn (H. Ota, unpublished information). The present field observations revealed substantial differences in live coloration between *G. k. orientalis* sensu stricto and the Tokashikijima population which is described above as *G. k. sengokui* subsp. nov.; *G. k. sengokui* subsp. nov. usually showed reddish or pinkish tint to some extent in dorsal pattern and iris irrespective of ages in life, whereas *G. k. orientalis* sensu stricto showed no such tint of coloration in corresponding portion in comparable condition (Figure 3).

Values for meristic characters examined here did not predict any utility of these characters for unequivocal discrimination of the two subspecies. In body size as represented by SVL, *G. k. sengokui* subsp. nov. may be largely or distinctly smaller than *G. k. orientalis* sensu stricto, but this obviously needs careful verification on the basis of additional data (Table 1).

Our previous molecular phylogenetic analyses of the *G. kuroiwa* species group suggested sister-group relationship of the Tokashikijima population (= *G. k. sengokui* subsp. nov.) with the Tonakijima population (= *G. k. orientalis* sensu stricto) (Honda *et al.*, 2014). Our recalculation of published sequence data confirmed substantial genetic differences of *G. k. sengokui* subsp. nov. from *G. k. orientalis*, or from any of the other taxa currently recognized in the Ryukyu Archipelago (Table 2). Also, relatively larger molecular divergences were observed between subspecies of *G. kuroiwa* including *G. k. sengokui* subsp. nov. compared to those between two species in the continent (*G. lui* and *G. huiliensis*: see Results). These results further support the validity of *G. k. sengokui* subsp. nov. as a distinguishable taxon. However, the degree of divergence alone should not be the standard for species-subspecies border (Torstrom *et al.*, 2014). The canonical discriminant analysis excluding coloration and banding pattern could not reveal morphological gaps among subspecies of *G. kuroiwa* (= “*kuroiwa*”, “*orientalis*”, “*yamashinae*” and “*toyamai*”), while demonstrating a clear morphological boundary between these four taxa and *G. splendens* (Grismer *et al.*, 1994). Phylogenetically, Honda *et al.* (2014) suggested a hierarchic structure of *G. splendens* against to the others. On the other hand, although Grismer *et al.* (1999) elevated five taxa in the Ryukyu Archipelago to full species from the viewpoint of evolutionary species concept, they did not discuss divergence nor phylogeny among them. We thus tentatively treat “*sengokui*” as subspecies, regarding

this diagnosable allopatric population as a sublineage of *G. kuroiwa*.

Besides the Tonakijima and Tokashikijima populations studied here, Akajima and Iejima populations have also been assigned to *G. k. orientalis* (Grismer *et al.*, 1994; Honda, 2002; Honda *et al.*, 2014; Ota *et al.*, 1999). To the present, however, Akajima population has been known only from one juvenile photographed and collected thereon in 1981 (Toyama, 1983), and the population is thus considered no more exist at all (Tanaka, 2005), most likely due to the predation pressure from the exotic weasel, which was artificially introduced to Akajima for rat control in the 1950s or 1960s (Uchida, 1969). Judging from external features including coloration of the unique representative specimen from Akajima (Grismer *et al.*, 1994; Toyama, 1983), as well as from close vicinity of Akajima with Tokashikijima (Figure 1) and their obvious dry-land connection during the Late Pleistocene Glacial Maxima (Ota and Masunaga, 2004), it is almost certain that the Akajima population also belonged to *G. k. sengokui* subsp. nov. in reality.

Much closer affinity of the Iejima population to an Okinawajima population of *G. k. kuroiwa* than to *G. k. orientalis* sensu stricto or *G. k. sengokui* subsp. nov. is evident from the results of molecular analyses (Honda *et al.* 2014: also see Table 2). Unfortunately, we have had no opportunities of examining live coloration or quantitative characters of the Iejima population in detail. However, as long as available information is concerned, this population may be discernible from *G. k. orientalis* and *G. k. sengokui* subsp. nov. in having six, instead of four or five, postmental scales (PM) touching the mental scale (Ota, 1989). The results of molecular analyses (Honda *et al.*, 2014; Ota *et al.*, 1999: also see Table 2) also support the divergence of the Iejima population from *G. k. orientalis* sensu stricto and *G. k. sengokui* subsp. nov. On the other hand, relatively large genetic divergence was observed between the southern and northern *G. k. kuroiwa* populations of Okinawajima (Honda *et al.*, 2014; Ota *et al.*, 1999: also see Table 2). Further studies on relevant populations using additional morphological and molecular data, those for nucleic DNAs in particular, are strongly desired to get to the convincing conclusions on the classification of *Goniurosaurus kuroiwa* sensu lato from the viewpoint of population systematics.

**Acknowledgements** We thank S. Kawada, J. Yamazaki, Y. Hamakawa, Y. Kobayashi, H. Wada, K. Yahata and members of the Graduate School of Life and Environmental Sciences, University of Tsukuba, for

helping and encouraging us throughout this project. Special thanks are due to S. Tanaka for providing us specimens from Tokashikijima. Our research was partially supported by a Grant-in-Aid from the Japan Ministry of Education, Culture, Sports, Science and Technology (Scientific Research C No. 22510244 to M. Honda), and by a grant from the Zoshinkai Fund for Protection of Endangered Animals (to H. Ota). All subspecies of *G. kuroiwa* are protected by the laws of Japanese and Okinawa Prefectural Governments. Sampling was conducted under permissions from Ministry of the Environment (Reference No. 1609203 to M. Honda, 2016–2017) and from the Okinawa Prefectural Board of Education (Reference Nos. 11 and 37 to M. Toda, 2009–2013, and No. 50 to M. Honda, 2016–2017).

## References

- Börner A. R.** 1981. The genera of Asian eublepharine geckos and a hypothesis on their phylogeny. *Misc Art in Sauroi*, 9: 1–14
- Dickhoff A.** 2004. Ein Tiger im Terrarium-Haltung und Nachzucht von *Goniurosaurus araneus* (Grismer, Viets & Boyle 1999). *Draco*, 5: 76–81
- Grismer L. L.** 1987. Evidence for the resurrection of *Goniurosaurus barbour* (Reptilia: Eublepharidae) with a discussion on geographic variation in *Goniurosaurus lichtenfelderi*. *Acta Herpetol Sinica*, 6: 43–47
- Grismer L. L.** 1988. The phylogeny, taxonomy, classification, and biogeography of eublepharid geckos (Reptilia: Squamata). In Estes R. (Ed.), *Phylogenetic Relationships of the Lizard Families*. California: Stanford University Press, 369–469
- Grismer L. L.** 2002. *Goniurosaurus*: ancient gekkos of the Far East. *Gekko*, 3: 22–28
- Grismer L. L., Ota H., Tanaka S.** 1994. Phylogeny, classification, and biogeography of *Goniurosaurus kuroiwa* (Squamata: Eublepharidae) from the Ryukyu Archipelago, Japan, with description of a new subspecies. *Zool Sci*, 11: 319–335
- Grismer L. L., Viets B. E., Boyle L. J.** 1999. Two new continental species of *Goniurosaurus* (Squamata: Eublepharidae) with a phylogeny and evolutionary classification of the genus. *J Herpetol*, 33: 382–393
- Honda M.** 2002. Conservation genetics of *Goniurosaurus kuroiwa*, with special reference to a population from the central part of Okinawajima Island, Ryukyu Archipelago. *Akamata*, (16): 15–18 (in Japanese)
- Honda M., Kurita T., Toda M., Ota H.** 2014. Phylogenetic relationships, genetic divergence, historical biogeography and conservation of an endangered gecko, *Goniurosaurus kuroiwa* (Squamata: Eublepharidae), from the Central Ryukyus, Japan. *Zoo Sci*, 31: 309–320
- Kurita T., Kawamura R.** 2011. New record of *Goniurosaurus kuroiwa* from Yagajijima Island of the Okinawa Group, Ryukyu Archipelago. *Akamata*, (22): 15–20 (in Japanese)
- Maenosono T., Toda M.** 2007. Distributions of amphibians and terrestrial reptiles in the Ryukyu Archipelago: a review of published record. *Akamata*, (18): 28–46 (in Japanese)
- Maki M.** 1930. A new banded gecko, *Eublepharis orientalis*, sp. nov. from Riu Kyu. *Annot Zool Jpn*, 13: 9–11
- Nakamura K., Uéno S. I.** 1963 *Japanese Reptiles and Amphibians in Color*. Osaka: Hoikusha
- Nakamura Y., Takahashi A., Ota H.** 2014. A new, recently extinct subspecies of the Kuroiwa's Leopard Gecko, *Goniurosaurus kuroiwa* (Squamata: Eublepharidae), from Yoronjima Island of the Ryukyu Archipelago, Japan. *Acta Herpetol*, 9: 61–73
- Okamoto T.** 2017. Historical biogeography of the terrestrial reptiles of Japan: a comparative analysis of geographic ranges and molecular phylogenies. In: Motokawa M., Kajihara H. (Eds.), *Species Diversity of Animals in Japan*. Tokyo: Springer Japan, 135–163
- Orlov N. L., Ryabov S. A., Nguyen T. T., Nguyen Q. T., Ho T. C.** 2008. A new species of *Goniurosaurus* (Sauria: Gekkota: Eublepharidae) from north Vietnam. *Russian J Herpetol*, 15: 229–244
- Ota H.** 1989. A review of the gecko (Scincidae: Lacertilia) of the Ryukyu Archipelago and Taiwan. In Matsui M., Hikida T., Goris R. C. (Eds.), *Current Herpetology in East Asia*. Kyoto: Herpetological Society of Japan, 222–261
- Ota H.** 1997. An annotated catalogue of type specimens of amphibians and reptiles in the National Science Museum, Tokyo. *Bull Nat Sci Mus Tokyo Ser A*, 23: 221–232
- Ota H.** 1998. Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications: *Res Popul Ecol*, 40: 189–204
- Ota, H.** 2010. *Goniurosaurus kuroiwa*. The IUCN Red List of Threatened Species 2010: e.T40793A10356760. <http://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T40793A10356760.en> (downloaded on 08 December 2016)
- Ota H., Masunaga G.** 2004. Herpetofauna of the Kerama Islands of the Okinawa Group, Ryukyu Archipelago, Japan. *Midori-ishi* (15): 29–35 (in Japanese)
- Ota H., Honda M., Kobayashi M., Sengoku S., Hikida T.** 1999. Phylogenetic relationships of eublepharid geckos (Reptilia: Squamata): a molecular approach. *Zool Sci*, 16: 659–666
- R Development Core Team.** 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>
- Sengoku S.** 1979. *Amphibians and Reptiles in Color*. Tokyo: Ienohikari-kyokai
- Seufer H., Kaverkin Y., Kirschner A.** 2005. *Die Lidgeckos*. Keltern-Weiler: Kirschner und Seuffer Verlag
- Tanaka S.** 1996. *Goniurosaurus kuroiwa*. In Sengoku S., Hikida T., Matsui M., Nakaya K. (Eds.), *The Encyclopedia of Animals in Japan*. Vol 5. Amphibians, Reptiles and Chondrichthyes. Tokyo: Heibonsha, 67–71 (in Japanese)
- Tanaka S.** 2005. *Goniurosaurus kuroiwa*. In Okinawa Prefectural Government, Nature Conservation Division, Department of Cultural and Environmental Affairs, Okinawa Prefectural Government (Ed.), *Threatened Wildlife in Okinawa 2nd Ed (Animals)*, Red Data Okinawa. Naha: Okinawa Prefectural Government, 138–140 (in Japanese)
- Torstrom S. M., Pangle K. L., Swanson B. J.** 2014. Shedding subspecies: The influence of genetics on reptile subspecies



- taxonomy. *Mol Phylogent Evol*, 76: 134–143
- Toyama M.** 1983. Preliminary reports on the herpetological fauna of the Okinawa Islands, Ryukyu Archipelago (II). *Bull Okinawa Pref Mus*, 7: 6–22 (in Japanese)
- Toyama M.** 1984. Amphibians and Reptiles. In Ikehara S., Yonashiro Y., Miyagi K., Toyama, M. (Eds.), *Handbook of the Animals of the Ryukyu Archipelago (I): Terrestrial Vertebrates*. Naha: Shinsea-tosho Shuppan, 208–352 (in Japanese)
- Toyama M., Ota H.** 1991. Amphibians and reptiles of the Ryukyu Islands. In *World Wildlife Fund, Japan (Ed.) Study of Essential Factors for Preservation of Wildlife in Nansei Islands*. Tokyo: Japan Environmental Agency, 233–254 (in Japanese)
- Uchida T.** 1969. Rat-control procedures on the Pacific island, with special reference to the efficiency of the biological control agents. II. Efficiency of the Japanese weasel, *Mustela sibirica itatsi* Temminck & Schlegel, as a rat-control agent in the Rryukyus. *Fac Agr Kyushu Univ*, 15: 355–385
- Wang Y. Y., Yang J. H., Cui R. F.** 2010. A new species of *Goniurosaurus* (Squamata: Eublepharidae) from Yingde, Guangdong Province, China. *Herpetologica*, 66: 229–240
- Wang Y. Y., Yang J. H., Grismer L. L.** 2013. A new species of *Goniurosaurus* (Squamata: Eublepharidae) from Libo, Guizhou Province, China. *Herpetologica*, 69: 214–226
- Wang Y. Y., Jin M. J., Li Y. L., Grismer L. L.** 2014. Description of a new species of *Goniurosaurus* (Squamata: Eublepharidae) from the Guangdong Province, China, based on molecular and morphological data. *Herpetologica*, 70: 309–322
- Wermuth H.** 1965. Gekkonidae, Pygopodidae, Xantusidae. In Mertens R., Hennig W., Wermuth H. (Eds.), *Das Tierreich Vol 80*. Berlin: Walter de Gruyter and Co., 154–155
- Werner Y. L., Takahashi H., Yasukawa Y., Ota H.** 2004. The varied foraging mode of the subtropical eublepharid gecko *Goniurosaurus kuroiwaie orientalis*. *J Nat Hist*, 38: 119–134
- Yang J. H., Chan B. P. L.** 2015. Two new species of the genus *Goniurosaurus* (Squamata: Sauria: Eublepharidae) from southern China. *Zootaxa*, 3980: 67–80
- Ziegler T., Nguyen Q. T., Schmitz A., Stenke R., Rösler H.** 2008. A new species of *Goniurosaurus* from Cat Ba Island, Hai Phong, northern Vietnam (Squamata: Eublepharidae). *Zootaxa*, 1771: 16–30